Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Decreased phosphatidylcholine plasmalogens – A putative novel lipid signature in patients with stable coronary artery disease and acute myocardial infarction

Iryna Sutter [a, b, 1], Roland Klingenberg [c, 1], Alaa Othman [a, e], Lucia Rohrer [a], Ulf Landmesser [b, c, d], Dierik Heg [e], Nicolas Rodondi [f], Francois Mach [g], Stephan Windecker [h], Christian M. Matter [b, c], Thomas F. Lüscher [b, c], Arnold von Eckardstein [a, b, i], Thorsten Hornemann [a, b, i, *]

[a] *Institute of Clinical Chemistry, University and University Hospital of Zurich, Zurich, Switzerland*
[b] *Competence Center for Integrated Human Physiology, University of Zurich, Zurich, Switzerland*
[c] *Department of Cardiology and Cardiovascular Research, University Heart Center, University Hospital Zurich, Zurich, University of Zurich, Switzerland*
[d] *Department of Cardiology, Charité Universitätsmedizin Berlin, Berlin, Germany*
[e] *Institute of Social and Preventive Medicine, Clinical Trials Unit, University of Bern, Bern, Switzerland*
[f] *Department of Internal Medicine, University Hospital Bern, Bern, Switzerland*
[g] *Cardiology, University Hospital Geneva, Switzerland*
[h] *Cardiovascular Center, University Hospital Bern, Switzerland*
[i] *Competence Center for Systems Physiology and Metabolic Diseases, ETH Zurich and University of Zurich, Zurich, Switzerland*

Keywords:
Glycerophospholipids
Sphingolipids
Coronary artery disease
Acute myocardial infarction

A B S T R A C T

Objective: Glycerophospholipids and sphingolipids are structurally heterogeneous due to differences in the O- and N-linked fatty acids and head groups. Sphingolipids also show a heterogeneity in their sphingoid base composition which up to now has been little appreciated. The aim of this study was to investigate the association of certain glycerophospholipid and sphingolipid species with stable coronary artery disease (CAD) and acute myocardial infarction (AMI).
Methods: The lipid profile in plasma from patients with stable CAD (n = 18) or AMI (n = 17) was compared to healthy subjects (n = 14). Sixty five glycerophospholipid and sphingolipid species were quantified by LC-MS. The relative distribution of these lipids into lipoprotein fractions was analyzed.
Results: In the CAD cohort, 45 glycerophospholipid and sphingolipid species were significantly lower compared to healthy controls. In the AMI group, 42 glycerophospholipid and sphingolipid species were reduced. Four PC plasmalogens (PC33:1, PC33:2, PC33:3 and PC35:3) showed the most significant difference. Out of eleven analyzed sphingoid bases, four were lower in the CAD and six in the AMI group. Sphingosine-1-phosphate (S1P) levels were reduced in the AMI group whereas an atypical C16:1 S1P was lower in both groups. Phosphatidylcholine and sphingomyelin species were exclusively present in lipoprotein particles, whereas lysophosphatidylcholines were mainly found in the lipoprotein-free fraction. The observed differences were not explained by the use of statins as confirmed in a second, independent cohort.
Conclusions: Reduced levels of four PC plasmalogens (PC33:1, PC33:2, PC33:3 and PC35:3) were identified as a putatively novel lipid signature for CAD and AMI.

1. Introduction

Coronary artery disease (CAD) is a major cause of death and morbidity in the western world [1]. CAD is caused by the gradual development of atherosclerotic plaques in the arterial wall. A rupture or erosion of plaques with subsequent total occlusion of an

* Corresponding author. Inst. for Clinical Chemistry, University Hospital Zurich, Raemistrasse 100, CH-8091, Zurich, Switzerland.
E-mail address: thorsten.hornemann@usz.ch (T. Hornemann).
[1] Both authors contributed equally.

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

epicardial coronary artery may lead to acute myocardial infarction (AMI) [2,3]. Recent data suggest that beyond established lipids risk factors like cholesterol and triacylglycerols also glycerophospholipids and sphingolipids contribute to atherogenesis [4,5]. Plasma glycerophospholipids and sphingolipids are mainly associated with lipoprotein particles [6–8] and form a lipid monolayer around the hydrophobic core of triacylglycerols and cholesteryl esters. Prior work identified distinct lipid profiles of plasma, serum or lipoprotein particles in patients with CAD [9–13]. Hundreds of different glycerophospholipid and sphingolipid species were identified in plasma lipoproteins and atherosclerotic plaques [14,15]. In particular sphingolipids are structurally diverse because of a multiplicity in O-linked head groups and N-linked fatty acids. Sphingolipids also show a significant heterogeneity in the sphingoid base composition which yet has been only little appreciated. Sphingolipid de-novo formation is initiated by the conjugation of L-serine and acyl-CoA, a reaction catalyzed by the enzyme serine palmitoyltransferase (SPT). Sphingoid bases, the product of this reaction are the common structural feature for all sphingolipid species. The most abundant sphingoid bases in plasma is C_{18} sphingosine, but SPT can also form other sphingoid bases in the range of C_{14}–C_{20} [16]. Under certain conditions, SPT also metabolizes L-alanine and glycine which forms an atypical category of 1-deoxysphingolipids (1-deoxySLs) [17,18] which were shown to be elevated in several metabolic conditions.

The aim of this study was to identify novel plasma lipid signatures for patients with CAD or AMI.

2. Materials and methods

2.1. Patients

Healthy subjects (n = 14) were recruited from the local blood bank. Exclusion criteria were the use of cardiovascular medication, positive cardiovascular family history, smoking (including cessation of smoking within 2 years prior to study enrollment), history of hypertension, elevated total cholesterol (≥5.0 mmol/L), BMI > 30 kg/m2, history of diabetes mellitus, evidence of relevant vascular or structural heart disease and/or a reduced LVEF (<55%) on echocardiogramm. The stable CAD group (n = 18) was selected from patients with angiographically documented coronary artery stenosis ≥50%. Exclusion criteria comprised an ACS within the preceding 6 months, systemic infectious, inflammatory or autoimmune disease, known severe renal dysfunction (serum creatinine >220 μmol/L), known severe hepatic dysfunction (3x ULN for LFTs), neoplasm or other life-threatening disease with a life expectancy less than one year, extended surgery in the preceding 3 months and/or evidence of vascular or structural heart disease and/or a reduced systolic LV function on echocardiogram or left ventricular angiogram.

Patients with AMI (n = 17) were selected from the Inflammation in Acute Coronary Syndromes cohort (SPUM-ACS, NCT01000701). SPUM-ACS inclusion criteria comprised both genders (aged ≥18 years) presenting within five days (preferably within 72 h) after onset of chest pain and a main diagnosis of STEMI, NSTEMI or unstable angina as described previously [19].

All patients gave written informed consent and the study was conducted with approval from the local ethics committee (EK-1680).

2.2. Sampling of plasma

Blood was drawn from the antecubital vein in healthy subjects and from the femoral/radial artery at the time of diagnostic coronary angiography in patients with CAD or AMI, respectively. Samples were centrifuged at 2700 *g* for 10 min at room temperature to obtain plasma, frozen and stored in aliquots at −80 °C until serial measurement (no prior freeze-thaw cycles). To avoid inter-assay differences all samples were extracted and analyzed in a single batch. The person who performed the analysis was blinded to the patient's data by the use of numbered ID codes.

2.3. Clinical chemistry

Plasma concentrations or activities of total cholesterol, triglycerides, LDL-C and HDL-C, glucose, creatine kinase, CK-MB, and troponin levels were determined by photometric tests or immunoassay by using the Cobas 8000 autoanalyzer from Roche diagnostics (Rotkreuz, Switzerland).

2.4. Isolation of lipoproteins

Human lipoprotein fractions and the lipoprotein-free fraction (LFF) were isolated from plasma of three healthy blood donors by stepwise ultracentrifugation, as described previously [20]. Purity of lipoprotein fractions was confirmed by SDS-PAGE and Coomassie Blue staining.

2.5. Analysis of plasma glycerophospholipids and sphingolipids

Six lipid classes including phosphatidylglycerols (PGs), phosphatidic acids (PAs), phosphatidylethanolamines (PEs), phosphatidylcholines (PCs), sphingomyelins (SMs) and ceramides/hexosylceramides (Cers/HexCers) were analyzed. Lipids were quantified in relation to internal standards (200 ng) including PG(17:0/17:0), LPG(17:1/0:0), PA(14:0/14:0), LPA(17:0/0:0), PE(14:0/14:0), LPE(17:1/0:0), PC(14:0/14:0), PC(24:0/24:0), LPC(17:0/0:0), SM (d18:1/12:0) and Cer(d18:1/17:0) (Avanti Polar Lipids, Alabaster, AL, USA). 20 μl plasma was extracted with 375 μl of methanol/chloroform (2:1 v/v). After vortexing, 100 μl water and 125 μl chloroform were added, agitated (15 min), centrifuged (16,100 ×*g* for 5 min at 25 °C) and the lower phase collected. Again 250 μl chloroform was added, agitated (15 min) and centrifuged. The lower phases were combined and evaporated under a stream of nitrogen. Analysis was done on a TSQ Quantum Access triple quadrupole connected to a Rheos 2200 pump. Separation was done on a diol silica-based column (QS Uptisphere 6 OH, 150 × 2.1 mm, 5 μm). Mobile phase (A) was hexane/isopropanol/water (70:30:2 v/v) including 15 mM NH_4COOH; and (B) isopropanol/water (50:2 v/v) including 15 mM NH_4COOH at a flow rate of 0.35 ml/min. Gradient was 0–7 min A/B (%) 80/20; 8–10 min A/B (%) 60/40, 11–23 min A/B (%) 40/60 and 25–30 min A/B (%) 80/20. Dried material was reconstituted in 200 μl of mobile phases A (80%) and B (20%) an injected (10 μl) into the LC-MS. Neutral loss and precursor scans were used to detect specific glycerophospholipids and sphingolipids. A neutral loss scan of *m/z* 115 and 189 from $[M + NH_4]^+$ ions was used for the analysis of PA and PG lipids. Precursor ion scan of *m/z* 184, specific for phosphocholine was used for PC, SM and LPC lipids. Neutral loss scan of *m/z* 141 was used for PEs and precursor scanning of *m/z* 264 was applied for Cer and HexCer species. Data were analyzed in Xcalibur (version 2.0.6, Thermo Scientific). Identification of molecular species was performed by lipid mass spectrum analysis software (LIMSA) [21].

The assignment of glycerophospholipids includes total numbers of carbons and double bonds in two acyl chains. The SM assignment comprises total numbers of carbons and double bonds in the sphingoid base and N-linked fatty acid. Hexosylceramide species include isomeric glucosyl- and galactosylceramides. For identified Cers and HexCers, molecular structures are reported. Quantification was done in relation to the respective internal standards.

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

2.6. Quantification of sphingosine-1-phosphate species

Sphingosine-1-phosphates (S1Ps) in plasma samples were determined by LC-MS as described previously [22]. Concentrations of S1P species were measured in 25 μl of plasma spiked with 10 pmol internal standard 18:1-D7S1P (Avanti Polar Lipids, Alabaster, AL, USA). Analysis of S1P species included the quantification of 16:1-S1P, 17:1-S1P, 18:1-S1P and 18:0-S1P lipids.

2.7. Quantification of sphingoid bases

The profile of 11 plasma sphingoid bases was analyzed by LC-MS as described earlier [23]. d7-sphingosine (D7SO) and d7-sphinganine (D7SA) (200 pmol each) were used as internal standards (Avanti Polar Lipids, Alabaster, AL, USA).

2.8. Statistical analysis

Statistical analysis was performed using SPSS, version 19 (IBM Corporation, Somers NY, USA). Normality of the data was determined by using the **Kolmogorov-Smirnov test**. For variables which did not show a normal distribution, univariate statistics was done using Kruskal-Wallis and the Mann-Whitney U tests. Categorical variables were compared using the Chi-square test. Spearman rank tests were used to describe the correlations of plasma glycerophospholipids and sphingolipids with total cholesterol, LDL-C and HDL-C levels. The *p* values were adjusted for multiple comparisons by applying the Benjamini-Hochberg procedure [24]. Adjusted (adj.) *p* values of <0.05 were considered statistically significant.

3. Results

3.1. Patient characteristics

Here we compared the plasma lipid profile between healthy subjects and patients with stable CAD (n = 18) or AMI (n = 17). The age of the patients ranged between 43 and 77 years. On average, healthy subjects (56.4 ± 7.5 years) were ten and patients with CAD (61.3 ± 8.3 years) five years younger than patients with AMI (66.8 ± 10.0 years). Features of acute myocardial infarction, including elevated levels of troponin T, NT-proBNP, creatine kinase and CK-MB were higher in the AMI group (Table 1). Prevalence of smoking was significantly higher in CAD and AMI group. Average BMI was higher in patients with CAD compared to healthy subjects. LDL-C and total cholesterol levels were lower in patients with stable CAD and AMI due to statin treatment.

3.2. Plasma lipidome – alterations in patients with CAD and AMI

Sixty-five lipid species were analyzed in this study. As statin use was significantly higher in the CAD and AMI group (Table 1) we first tested for an influence of statins on the analyzed lipids species by comparing CAD patients with and without statin treatment (Table 2). No significant differences for the analyzed glycerophospholipid and sphingolipid species were observed between the two groups.

Next we compared the same lipid profile between healthy subjects and patients with CAD and AMI. Univariate statistics revealed significant changes in the plasma lipid profile for patients with stable CAD and AMI compared to healthy individuals (Table 3). The total levels of PC, LPC, PE, SM and Cer/HexCer lipids were generally lower in patients with stable CAD and AMI compared to healthy subjects (Table 3). Total Cer and HexCer were further reduced in patients with AMI compared to the CAD group. Comparing the individual lipid species we observed differences in 45 molecular species (19 PCs, 4 LPCs, 5 PEs, 12 SMs and 5 Cers/HexCers) for the CAD and differences in 42 lipid species (15 PCs, 2 LPCs, 9 PEs, 11 SMs and 5 Cers/HexCers) for the AMI group in comparison to healthy subjects (Table 3).

Sphingoid bases are the shared structural component for all sphingolipids. C18 sphingosine (d18:1) is typically the most abundant sphingoid base in plasma although a wide range of other sphingoid bases can be found too. For sphingoid base analysis, total sphingolipids were extracted from plasma samples and hydrolyzed to remove the conjugated N-acyl chain and head groups. It is important to note that the here reported sphingoid base concentrations refer to the total levels of a given sphingoid base in plasma irrespective of the sphingolipid specie where it was originally contained (e.g Cer, hexCer, SM etc). In the CAD and AMI group, we observe lower plasma levels for C_{17}SO, C_{18}SO, C_{18}SA and C_{18}SAdiene

Table 1
Clinical characteristics of the study population. Values are means ± SD or percentages for scale or categorical variables. Statistical significance was determined by the Kruskal-Wallis (multiple group comparisons), and by the Mann-Whitney U test (two group comparisons). p values for the categorical variables were calculated by using Chi-square test. p values were adjusted for multiple testing by using the Benjamini-Hochberg procedure. (Significant results are marked bold,* variables were measured before hospital discharge).

Characteristics	Healthy subjects (n = 14)	CAD (n = 18)	AMI (n = 17)	Kruskal-Wallis, adj. *p* Values	Mann-Whitney U, adj. *p* Values		
	Mean ± SD	Mean ± SD	Mean ± SD		H vs CAD	H vs ACS	CAD vs ACS
Age (years)	56.4 ± 7.5	61.3 ± 8.3	66.8 ± 10.0	**0.005**	0.164	**0.004**	0.084
Gender, female (%)	36	22	18	0.488	0.492	0.347	0.784
Smoking (%)	0	75	56	**0.001**	**<0.001**	**0.04**	0.352
Statin treatment (%)	0	100	100	**<0.001**	**<0.001**	**<0.001**	0.649
BMI (kg/m2)	24.5 ± 3.48	27.85 ± 3.52	26.45 ± 3.15	**0.03**	**0.035**	0.084	0.22
Systolic blood pressure (mmHg)	119.36 ± 13.99	129.89 ± 16.52	128.59 ± 19.11	0.248	0.163	0.232	0.92
Diastolic blood pressure (mmHg)	71.36 ± 9.79	76.50 ± 10.76	76.06 ± 11.27	0.267	0.22	0.215	0.981
Glucose (mmol/l)	5.18 ± 0.44	5.43 ± 0.56	6.32 ± 0.98	**0.005**	0.362	**0.006**	**0.018**
Total cholesterol (mmol/l)	5.78 ± 0.97	4.05 ± 0.89	3.84 ± 1.00	**<0.001**	**<0.001**	**<0.001**	0.448
LDL-C (mmol/l)	3.53 ± 0.83	2.39 ± 0.76	2.28 ± 0.92	**0.001**	**0.004**	**0.004**	0.609
HDL-C (mmol/l)	1.83 ± 0.42	1.18 ± 0.32	1.22 ± 0.28	**<0.001**	**<0.001**	**0.001**	0.618
Triglycerides (mmol/l)	0.98 ± 0.43	1.07 ± 0.46	0.75 ± 0.53	0.099	0.738	0.132	0.081
NT-proBNP (ng/L)	41.21 ± 35.36	140.22 ± 108.47	817.88 ± 1013.81	**<0.001**	**0.006**	**<0.001**	**0.026**
Creatine kinase (μg/l)	136.18 ± 76.96	119.65 ± 42.47	1304.76 ± 828.56	**<0.001**	0.981	**<0.001**	**<0.001**
CK-MB (μg/l)*	11.60 ± 3.47	10.86 ± 5.41	141.49 ± 103.43	**<0.001**	0.558	**<0.001**	**<0.001**
Troponin T (ug/l)*	0.01 ± 0	0.03 ± 0.03	2.65 ± 1.89	**<0.001**	**0.013**	**<0.001**	**<0.001**

Abbreviations: AMI, acute myocardial infarction; CAD, coronary artery disease; CK-MB, creatine kinase-MB; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Table 2
Plasma glycerophospholipids and sphingolipids in statin-treated and non-treated patients with CAD. Statistical significance was calculated by the Mann-Whitney U test. The p values were adjusted for multiple testing using the Benjamini-Hochberg procedure. Total lipids are presented for phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs), phosphatidylethanolamines (PEs), phosphatidylglycerols (PGs), sphingomyelins (SMs), ceramide/hexosylceramides (Cers/HexCers). Glycerophospholipids are shown with total numbers of carbons and double bounds. SM species are summarized by numbers of carbons and double bounds in sphingoid base and N-linked fatty acid. Sphingosine-1-phosphate (S1P) species include numbers of carbons and double bonds in sphingoid base. HexCer species include isobaric glucosyl- and galactosylceramides. (n.d. - not detected).

Lipids (μM)	CAD statin-treated (n = 10)	CAD statin-untreated (n = 10)	Mann-Whitney U, adj. *p* values
	Median(Min; Max)	Median(Min; Max)	CAD statin-treated vs CAD statin-untreated
Total PC	795.3(614.8; 895.6)	823.6(692.2; 1195.8)	0.649
PC32:0	5.6(3.1; 7.8)	6.8(4.6; 9.5)	0.406
PC32:1	8.5(3; 15.5)	11.6(3.6; 15.4)	0.649
PC33:1	5.3(2.4; 9)	6.7(4.3; 9.3)	0.402
PC33:2	5.4(3.3; 12.3)	7(3.7; 10.5)	0.402
PC33:3	2.8(1.4; 5.9)	3.5(2.5; 7.8)	0.48
PC34:0	7.5(4.4; 11.7)	9.1(5; 11.6)	0.553
PC34:1	87.4(50; 126.5)	112.9(77.4; 152.8)	0.402
PC34:2	152.2(88; 222.1)	174.5(115.6; 297.4)	0.649
PC34:3	6.3(4.3; 9)	7.3(3.9; 10.6)	0.498
PC35:1	2.7(1; 3.5)	3.8(1.9; 5.5)	0.402
PC35:2	5.8(3.5; 6.3)	6.9(4.7; 11.2)	0.465
PC35:3	4.4(2.3; 8)	5.6(3.9; 9.7)	0.429
PC35:4	11.6(8.9; 17.9)	14.9(8.6; 19.3)	0.48
PC35:5	7.8(4.8; 15.3)	9.3(6.8; 12.9)	0.649
PC36:0	4(2.8; 5.7)	3.9(1.8; 6.6)	0.837
PC36:1	22.6(16.8; 31.2)	31.1(18.9; 42.7)	0.402
PC36:2	81.8(56.3; 115.2)	86.4(67.2; 144.3)	0.702
PC36:3	53(41.6; 68.1)	54.9(38; 77.5)	0.869
PC36:4	81.8(47.4; 103.9)	74.5(53.9; 120.8)	0.869
PC36:5	8.6(2; 19.8)	9.9(6.2; 15.6)	0.759
PC37:4	10.3(7.2; 15.9)	11.6(7.3; 14.2)	0.649
PC37:5	12.5(7.8; 16.4)	15.2(9.3; 21.6)	0.465
PC37:6	5.3(4.1; 9)	6.7(4.6; 9.9)	0.406
PC38:3	27.7(18.9; 37.1)	24.1(20.4; 43.3)	0.9
PC38:4	65.1(28.3; 75.7)	55.8(37.7; 101.3)	0.735
PC38:5	23.3(12.3; 30)	21.8(17.3; 27)	0.735
PC38:6	24.9(10.4; 34.5)	24(15.5; 31.5)	0.9
PC40:5	6.1(4.3; 7.4)	6.1(4.5; 9.6)	0.848
PC40:6	10.6(5; 14.1)	10.3(5.7; 15.7)	0.869
Total LPC	123.6(80.5; 154.7)	129.3(88.7; 179.2)	0.735
LPC16:0	67.4(44.8; 77.5)	75.2(53; 96.7)	0.48
LPC18:0	21.3(14.8; 27.7)	25.8(12; 34)	0.649
LPC18:1	14.7(8.8; 20.4)	14(9; 26.2)	0.702
LPC18:2	20.6(8.8; 37.9)	15.1(9.1; 32.3)	0.649
Total PE	14.2(6.3; 40.2)	16.3(8.2; 70)	0.759
PE34:2	0.9(0.2; 3.3)	1.6(0.3; 5.5)	0.649
PE35:2	0.1(0.03; 0.3)	0.3(0.1; 0.6)	0.402
PE36:4	1.3(0.8; 3.4)	1.7(0.5; 6.2)	0.837
PE36:3	0.8(0.1; 1.7)	0.9(0.2; 4.5)	0.759
PE36:2	2.8(0.7; 8.7)	3.5(0.6; 20.6)	0.759
PE36:1	0.6(0.1; 2.5)	0.8(0.1; 5.3)	0.56
PE37:5	0.4(0.03; 0.5)	0.8(0.2; 1.3)	0.402
PE37:4	0.2(0.1; 0.6)	0.2(0.1; 0.5)	0.848
PE38:6	2(0.5; 7.3)	2.2(0.9; 7.4)	0.702
PE38:5	1(0.6; 2.5)	1.3(0.5; 3.2)	0.804
PE38:4	3.9(1.9; 10.4)	4.4(2.1; 15.6)	0.804
Total PG	0.4(0.2; 0.7)	0.4(0.2; 1.7)	0.94
Total PA	n.d.	n.d.	–
Total SM	502.6(283.2; 628)	560.4(428.8; 702)	0.465
SM32:1	11.1(5.5; 18)	14.6(12; 17.7)	0.402
SM34:0	4(1.1; 5)	4.4(1.5; 6.1)	0.694
SM34:1	100.3(62.1; 136)	115.8(86.2; 161.1)	0.402
SM34:2	14.6(8.5; 22.8)	16.2(12.5; 28.3)	0.498
SM36:1	29.9(15.6; 42)	26.3(22.9; 46.6)	0.94
SM36:2	10.3(7.1; 14.3)	11.4(8.1; 16.8)	0.735
SM38:1	26.7(15; 32.1)	29.4(21.5; 36.6)	0.465
SM39:1	9.9(5.2; 16.7)	10.9(6.9; 15)	0.702
SM39:2	1.3(0.8; 2.3)	1.5(0.7; 1.9)	0.694
SM40:1	53.7(28.3; 66)	67.5(42.8; 95)	0.406
SM40:2	31.9(19; 39.5)	38.9(24.7; 49.5)	0.402
SM41:1	26.2(11.1; 38)	34.7(23.7; 42.7)	0.406
SM41:2	17.1(8.8; 25.6)	21.9(14.3; 31.2)	0.618
SM42:1	43(20.7; 52.3)	48.7(33.7; 75.6)	0.649
SM42:2	74.3(53.1; 113.7)	88.1(68.1; 123.5)	0.56

(*continued on next page*)

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Table 2 (*continued*)

Lipids (μM)	CAD statin-treated (n = 10)	CAD statin-untreated (n = 10)	Mann-Whitney U, adj. *p* values
	Median(Min; Max)	Median(Min; Max)	CAD statin-treated vs CAD statin-untreated
SM42:3	28.9(21.3; 39.5)	34(19.9; 48.5)	0.702
Total Cer/HexCer	7.6(4.6; 13.4)	9.4(6.5; 21.5)	0.465
Cer(d18:1/24:0)	2.3(1.5; 3.5)	2.6(1.4; 5.4)	0.837
HexCer(d18:1/22:0)	1.4(0.8; 2.1)	1.3(0.5; 5.3)	0.848
HexCer(d18:1/23:0)	0.6(0.1; 1.5)	1.4(0.6; 2.4)	0.402
HexCer(d18:1/24:0)	2(1.1; 3.7)	2.8(1.4; 6.5)	0.429
HexCer(d18:1/24:1)	1.2(0.6; 2.6)	1.7(0.7; 2.9)	0.649
S1P species			
16:1-S1P	0.1(0.07; 0.15)	0.11(0.07; 0.13)	0.649
17:1-S1P	n.d.	n.d.	–
18:1-S1P	0.45(0.39; 0.64)	0.55(0.51; 0.78)	0.402
18:0-S1P	0.08(0.04; 0.15)	0.06(0.03; 0.1)	0.649
Sphingoid bases of sphingolipids			
C16SO	21.96(10.47; 29.5)	23.45(13.69; 27.98)	0.735
C16SA	0.56(0.25; 0.99)	0.55(0.27; 0.8)	0.9
C17SO	9.09(4.43; 10.95)	10.31(6.92; 14.97)	0.406
C18SO	89.08(57.3; 122.08)	112.52(79.23; 140.89)	0.498
C18SA	3.29(1.4; 5.36)	3.35(1.85; 6.49)	0.848
C18SAdiene	34.81(24.08; 46.54)	41.76(25.29; 51.8)	0.402
C19SO	3.56(1.71; 6.75)	3.34(1.73; 4.68)	0.869
C20SO	0.23(0.2; 0.37)	0.22(0.18; 0.34)	0.735
C20SA	0.04(0.02; 0.06)	0.04(0.02; 0.06)	0.848
doxSO	0.18(0.09; 0.39)	0.18(0.04; 0.4)	0.735
doxSA	0.09(0.04; 0.18)	0.09(0.02; 0.17)	0.735

based sphingolipids compared to healthy subjects but no difference between patients with CAD and AMI (Table 3). Plasma levels of C16SO and C16SA were significantly lower in patients with AMI compared to healthy controls (Table 3). 1-Deoxysphingoid bases were not significantly altered between the groups.

The analysis of S1P species showed the presence of canonical 18:1-S1P but also of other S1P species, including 16:1-S1P, 17:1-S1P and 18:0-S1P. Canonical (18:1) S1P was lower in patients with AMI compared to healthy individuals (Table 3) whereas an atypical 16:1-S1P was lower in both groups (Table 3). Also 17:1-S1P was detectable but levels were below the limit of quantification (0.04 μM).

For further analysis, lipid and clinical data were arranged as volcano plots, showing the relative difference (x-axes) versus significance (y-axes). The analyzed lipid species were divided into four groups: 1. glycerophospholipids including even-chain PCs, PEs, LPCs and PGs; 2. glycerophospholipids including odd-chain PCs; 3. sphingolipids including SMs, Cers/HexCers, S1Ps and sphingoid bases and 4. clinical measures. Comparisons of healthy subjects vs. patients with CAD are shown in Fig. 1A, comparisons of healthy subjects vs. patients with AMI in Fig. 1B, and comparisons of CAD vs. AMI in Fig. 1C. The analysis revealed four odd-chain PC species (PC33:1, PC33:2, PC33:3 and PC35:3) to be the most significantly lowered species in patients with CAD compared to healthy subjects (Fig. 1A). The same odd-chain PC species were found to be lowered in the AMI group (Fig. 1B). Comparing CAD and AMI did not reveal differences in the lipid species but differences in some clinical variables including troponin, creatine kinase and CK-MB (Fig. 1C).

3.3. Structural analysis identifies odd-chain phosphatidylcholines as PC plasmalogens

The four identified odd-chain PC species (PC33:1, PC33:2, PC33:3 and PC35:3) could be either PCs with an odd number of carbons or PC plasmalogens with a vinyl-ether bond at the sn-1 position of the glycerol backbone. Comparing the MS/MS fragmentation pattern of these species to a commercial standard we already identified PC35:3 as a PC(P-18:0/18:2) plasmalogen in a different study [13]. A similar structural analysis of the PC33:1, PC33:2 and PC33:3 species confirmed them as plasmalogens PC(P-18:0/16:0), PC(P-16:0/18:1) and PC(P-16:0/18:2), respectively. The identity of other odd-chain PCs species in the dataset was not further investigated.

3.4. Plasma glycerophospholipids and sphingolipids correlate with cholesterol levels

Most of the identified glycerophospholipids and sphingolipids showed a significant correlation with total cholesterol and LDL-C (Fig. 2). Correlation with HDL-C was also seen for most species but less pronounced. Among all glycerophospholipid and sphingolipid species, PC33:1, PC33:2, PC33:3 and PC35:3 showed the strongest correlation with HDL-C ($r = 0.81$, $r = 0.71$, $r = 0.75$ and $r = 0.68$, respectively). Furthermore, total cholesterol and LDL-C correlated strongly with the sphingoid bases C18SO ($r = 0.93$ and $r = 0.86$, respectively) and C17SO ($r = 0.85$ and $r = 0.81$, respectively). Saturated LPC species correlated more with total cholesterol and LDL-C than with HDL-C.

3.5. Glycerophospholipid and sphingolipid levels in the lipoprotein fractions

Because of the strong correlations between the individual lipoproteins and plasma glycerophospholipids and sphingolipid species, we analyzed the distribution of these lipids across the major lipoprotein fractions. Lipoproteins were isolated from plasma of three healthy volunteers and the lipid profile analyzed in Chylomicrons/VLDL, LDL, HDL and in the lipoprotein-free fraction (LFF). Species for PC, SM and LPC were detected in all plasma lipoprotein fractions. PE and Cer/HexCer species were also present in lipoproteins, but at levels below the limit of quantification. Neither PG nor PA lipids were identified in the lipoprotein or LFF fractions.

The relative percentages of total PC, SM and LPC lipids in the individual lipoprotein fractions are shown in Fig. 3A–C. The percentages of the individual PC, SM and LPC species relative to the total concentration of a given species in all lipoprotein fractions is

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Table 3
Comparison of plasma glycerophospholipids and sphingolipids in healthy subjects and patients with CAD and AMI (median with ranges). Statistical significance was calculated by Kruskal-Wallis and Mann-Whitney U. Statistically significant results are indicated in bold. p values were adjusted for multiple testing by the Benjamini-Hochberg procedure. (AMI acute myocardial infarction; CAD, coronary artery disease; n.d., not detected).

Lipids, μmol/l	Healthy subjects (n = 14)	CAD (n = 18)	AMI (n = 17)	Kruskal-Wallis, adj. *p* values	Mann-Whitney U, adj. *p* values		
	Median(Min; Max)	Median(Min; Max)	Median(Min; Max)		H vs CAD	H vs ACS	CAD vs ACS
Total PC	829.3(692.3; 974.1)	725.6(522.7; 793.4)	670.4(595.9; 868.8)	**<0.001**	**0.002**	**0.001**	0.38
PC32:0	6.8(5; 9.3)	5(3.3; 6.8)	5.6(3.5; 7.9)	**0.002**	**0.002**	**0.02**	0.314
PC32:1	9(4.9; 18.4)	6.2(3.2; 12)	7.3(4.2; 16.6)	0.094	0.05	0.2	0.697
PC33:1	7.7(7; 11.7)	5.5(2.5; 6.7)	5.8(3.9; 7.9)	**<0.001**	**<0.001**	**0.001**	0.584
PC33:2	10.1(6.7; 16)	5.9(2.6; 7.3)	6.4(4.3; 8.8)	**<0.001**	**<0.001**	**0.001**	0.314
PC33:3	5.6(3.6; 10.7)	2.8(1.7; 4.2)	3.2(1.6; 4.7)	**<0.001**	**<0.001**	**<0.001**	0.584
PC34:0	9.3(6.3; 12.7)	7.1(4.3; 10.5)	7.4(5.4; 9.2)	**0.004**	**0.015**	**0.002**	0.877
PC34:1	92.6(74.9; 122.2)	76.2(42; 101.3)	81.7(51.7; 101.2)	**0.043**	**0.026**	0.108	0.681
PC34:2	187.8(156.6; 226.7)	135.9(72.2; 181.7)	129.5(98; 150.1)	**<0.001**	**<0.001**	**<0.001**	0.471
PC34:3	8.7(5.1; 9.7)	5.5(3.6; 8.1)	4.5(3.2; 6.8)	**<0.001**	**0.002**	**0.001**	0.391
PC35:1	10.3(8; 20.6)	7.4(3.5; 13.1)	7.5(5.8; 12.3)	**0.004**	**0.006**	**0.006**	0.863
PC35:2	8.1(7; 11.4)	6.3(2.9; 8.8)	5.9(4.2; 9.3)	**<0.001**	**0.001**	**0.002**	0.681
PC35:3	7.5(6; 11)	5.1(2.3; 7.4)	5.1(2.7; 6.9)	**<0.001**	**<0.001**	**<0.001**	0.932
PC35:4	15.4(8.7; 22.6)	13(7.4; 15.8)	13.1(7.6; 20.7)	**0.031**	**0.015**	0.116	0.644
PC35:5	11.9(6.1; 21)	8.4(4.4; 11.2)	9.2(4.7; 17.6)	**0.041**	**0.024**	0.148	0.482
PC36:0	5.3(3.8; 7.7)	4(2.6; 5.4)	4(2.4; 6.7)	**0.005**	**0.003**	**0.022**	0.877
PC36:1	32.7(23.9; 38.3)	22.7(13.4; 30.5)	22.7(17.1; 33.3)	**<0.001**	**<0.001**	**0.002**	0.681
PC36:2	106.7(77.7; 124.3)	78.6(51.9; 112.1)	72.1(52.8; 87.3)	**<0.001**	**0.001**	**<0.001**	0.509
PC36:3	48.7(44; 68.8)	44.7(29.7; 57.6)	40.7(29.8; 47.2)	**0.001**	0.09	**0.001**	0.175
PC36:4	70(39.4; 84.3)	73.7(44; 102)	74.4(47.4; 89.1)	0.682	0.506	0.595	0.932
PC36:5	8.8(5.8; 21.7)	8.8(1.3; 14.9)	9.2(2; 22.7)	0.725	0.491	0.877	0.768
PC37:4	12.7(8.3; 17)	11.4(6; 14.1)	10.8(7.3; 15.3)	0.226	0.235	0.159	0.863
PC37:5	17.3(11; 23.1)	13.5(7.7; 18.6)	14.5(9; 22.5)	**0.026**	**0.018**	0.085	0.509
PC37:6	8.6(5.9; 14.9)	6.2(3.4; 8)	7(3.3; 11.6)	**0.003**	**0.002**	**0.028**	0.286
PC38:3	21.6(11.2; 31.9)	23.5(14.4; 38.5)	19.8(10.9; 28.6)	0.157	0.506	0.386	0.091
PC38:4	49.5(28.9; 70.1)	60.7(41.5; 77.5)	60.6(38.4; 72.8)	0.091	**0.047**	0.29	0.482
PC38:5	19.3(11.8; 26.7)	18.3(8.2; 28.1)	18.9(12.2; 27.7)	0.979	0.95	0.877	0.863
PC38:6	17(12.7; 34.3)	20.7(9.3; 32.4)	18.7(11.1; 34.6)	0.259	0.148	0.815	0.38
PC40:5	5.3(3.3; 7.1)	5.9(3.5; 8.8)	5(2.8; 9.2)	0.387	0.34	0.904	0.343
PC40:6	7.7(5.1; 14.1)	10(5.5; 14.2)	8.5(5.7; 16.1)	0.201	0.104	0.506	0.482
Total LPC	179.2(156.8; 245.2)	136.3(105.3; 181.5)	136.6(110.5; 199.9)	**<0.001**	**0.001**	**0.004**	0.623
LPC16:0	90.8(77.4; 121.1)	72.5(52.3; 95.9)	64.1(49.6; 104.2)	**<0.001**	**0.001**	**0.001**	0.235
LPC18:0	30.7(26.4; 46.7)	25.6(20; 34.1)	22(15.7; 33.3)	**0.001**	**0.006**	**0.002**	0.098
LPC18:1	21.3(15.4; 28.5)	15.6(10.6; 21.8)	21.2(9.1; 27.6)	**0.004**	**0.004**	0.572	**0.022**
LPC18:2	32.5(25.6; 50.6)	19.5(9.6; 50)	32.1(11.6; 71.6)	**0.005**	**0.005**	0.67	**0.024**
Total PE	16.3(9.9; 26)	11.2(3.4; 23.4)	9.4(5; 17.6)	**0.003**	**0.028**	**0.003**	0.26
PE34:2	1.7(0.9; 3.2)	0.7(0.1; 2.3)	0.6(0.3; 1.8)	**0.001**	**0.003**	**0.001**	0.697
PE35:2	0.3(0.1; 0.5)	0.1(0.03; 0.3)	0.1(0.01; 0.2)	**<0.001**	**0.002**	**0.001**	0.768
PE36:4	1.4(0.8; 2.7)	1(0.3; 2.3)	0.9(0.4; 2)	0.07	0.11	**0.049**	0.721
PE36:3	1.1(0.5; 3.1)	0.6(0.02; 1.3)	0.5(0.2; 0.8)	**0.001**	**0.006**	**0.002**	0.605
PE36:2	4(2.1; 8.5)	1.9(0.4; 5.1)	1.6(0.5; 3.4)	**<0.001**	**0.005**	**0.001**	0.248
PE36:1	0.7(0.2; 2)	0.5(0.05; 1.1)	0.4(0.1; 1)	**0.041**	0.065	**0.049**	0.391
PE37:5	0.7(0.4; 1.1)	0.4(0.1; 0.7)	0.4(0.2; 1)	**0.004**	**0.003**	**0.015**	0.79
PE37:4	0.4(0.2; 0.8)	0.3(0.02; 0.6)	0.2(0.1; 0.4)	**0.014**	0.069	**0.006**	0.623
PE38:6	1.6(0.5; 3.7)	1.6(0.4; 4)	1.1(0.5; 2.9)	0.401	0.857	0.276	0.449
PE38:5	1.1(0.4; 2.6)	0.8(0.3; 2)	0.8(0.4; 1.8)	0.068	0.069	0.065	0.932
PE38:4	2.9(1.9; 6.3)	2.9(1; 5.5)	2.3(1.3; 4.9)	0.055	0.811	**0.049**	0.079
Total PG	0.3(0.1; 0.8)	0.3(0.1; 0.7)	0.2(0.1; 0.6)	0.099	0.543	0.108	0.117
Total PA	n.d.	n.d.	n.d.	-	-	-	–
Total SM	462.9(359.6; 600.4)	385.4(262.7; 547.9)	355.4(275.6; 475.7)	**0.002**	**0.007**	**0.002**	0.523
SM32:1	16.1(12.2; 22.6)	10.7(5.3; 17.8)	10.7(5.3; 16.4)	**0.001**	**0.006**	**0.001**	0.972
SM34:0	4.8(3.1; 6.1)	2.9(0.3; 4.5)	2.7(1.1; 4.2)	**0.001**	**0.003**	**0.002**	0.863
SM34:1	123.6(88.3; 169.2)	99.3(63; 131)	90.6(63.8; 127.6)	**<0.001**	**0.002**	**0.001**	0.41
SM34:2	17.8(13; 23.5)	14.6(7.8; 27.8)	13.2(10.8; 20.1)	**0.017**	**0.043**	**0.012**	0.823
SM36:1	23.4(16.4; 33.5)	22(14.8; 32.3)	19.6(11.9; 26.4)	0.345	0.523	0.185	0.623
SM36:2	9.6(7.4; 14.7)	8.8(6.4; 18.8)	8.5(4.9; 13.1)	0.361	0.652	0.231	0.482
SM38:1	23.2(15.8; 28.2)	17.9(12.3; 26.5)	16.9(12.2; 21.7)	**0.006**	**0.04**	**0.006**	0.286
SM39:1	9.2(5.9; 11.7)	6.8(4.3; 11.3)	5.6(4.3; 10.6)	**0.005**	**0.028**	**0.004**	0.499
SM39:2	1.2(0.5; 2.2)	1(0.3; 2.2)	0.9(0.3; 1.8)	0.371	0.435	0.29	0.566
SM40:1	42.6(31.7; 59.8)	33.7(25.9; 47.4)	32(22.2; 44.8)	**0.004**	**0.015**	**0.004**	0.697
SM40:2	28(18.6; 35.8)	23.1(16.4; 36.1)	21(16.9; 37.2)	**0.017**	**0.026**	**0.022**	0.681
SM41:1	22.2(17; 32.9)	19.3(9.7; 28.3)	15.4(13.3; 21.9)	**0.001**	**0.016**	**0.001**	0.314
SM41:2	14(11.6; 23.1)	12(7.3; 17.3)	11.8(8; 20.6)	**0.049**	**0.043**	0.065	0.823
SM42:1	39.6(25.8; 53.1)	29.8(19; 42.5)	25.8(19.1; 40.2)	**0.005**	**0.03**	**0.005**	0.327
SM42:2	66.4(51.6; 82.8)	53.6(40.9; 77.6)	55.3(38.4; 79.6)	**0.016**	**0.018**	**0.026**	0.823
SM42:3	22.8(16.7; 33.4)	21.4(15.3; 30.9)	21.5(13.6; 36.8)	0.38	0.324	0.326	0.811
Total Cer/HexCer	12.2(4.9; 20.2)	8.5(2.8; 11.7)	5.4(3.1; 7.4)	**<0.001**	**0.013**	**0.001**	**0.026**
Cer(d18:1/24:0)	2.2(1.4; 4.9)	1.7(1; 2.6)	1.6(0.7; 2.7)	**0.041**	**0.04**	**0.049**	0.932
HexCer(d18:1/22:0)	2.8(0.9; 5)	1.9(0.3; 3)	1.1(0.3; 1.7)	**<0.001**	**0.033**	**0.001**	**0.01**
HexCer(d18:1/23:0)	1.5(0.5; 2.9)	0.8(0.2; 1.7)	0.6(0.2; 1.1)	**0.001**	**0.016**	**0.001**	0.117

(*continued on next page*)

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Table 3 (*continued*)

Lipids, µmol/l	Healthy subjects (n = 14)	CAD (n = 18)	AMI (n = 17)	Kruskal-Wallis, adj. *p* values	Mann-Whitney U, adj. *p* values		
	Median(Min; Max)	Median(Min; Max)	Median(Min; Max)		H vs CAD	H vs ACS	CAD vs ACS
HexCer(d18:1/24:0)	3.5(1.6; 5.7)	2.2(0.3; 3.8)	1.3(0.7; 2.3)	**<0.001**	**0.012**	**<0.001**	**0.022**
HexCer(d18:1/24:1)	2.2(0.5; 2.9)	1.3(0.5; 2.5)	0.9(0.3; 1.7)	**0.004**	**0.04**	**0.004**	0.185
S1P species							
16:1-S1P	0.12(0.09; 0.15)	0.1(0.05; 0.17)	0.1(0.07; 0.13)	**0.006**	**0.014**	**0.009**	0.952
17:1-S1P	0.03(0.02; 0.05)	n.d.	n.d.	-	-	-	-
18:1-S1P	0.55(0.5; 0.81)	0.5(0.38; 0.82)	0.44(0.29; 0.79)	**0.011**	0.096	**0.013**	0.11
18:0-S1P	0.05(0.03; 0.07)	0.04(0.03; 0.09)	0.03(0.02; 0.14)	0.198	0.248	0.159	0.523
Sphingoid bases of sphingolipids							
C16SO	31.91(14.84; 46.01)	24.97(13.24; 58.88)	21.05(9.48; 48.36)	**0.011**	0.06	**0.004**	0.78
C16SA	0.73(0.3; 1.38)	0.63(0.21; 1.26)	0.46(0.24; 1.13)	**0.011**	0.263	**0.006**	0.139
C17SO	13.49(8.45; 22.2)	9.65(3.43; 17.94)	9(4.75; 23.95)	**0.003**	**0.016**	**0.003**	0.485
C18SO	145.13(104.41; 203.95)	110.45(64.43; 177.81)	105.05(67.5; 160.82)	**<0.001**	**0.002**	**0.001**	0.475
C18SA	4.5(3.11; 6.77)	3.2(1.28; 10.65)	3.02(1.68; 9.48)	**0.001**	**0.012**	**0.001**	0.544
C18SAdiene	52.44(30.66; 78.94)	37.15(24.66; 74.43)	35.66(23.3; 48.22)	**0.005**	**0.043**	**0.003**	0.501
C19SO	3.79(1.66; 7.28)	4.09(1.33; 8.66)	3.12(1.2; 6.81)	0.378	0.932	0.269	0.386
C20SO	0.24(0.14; 0.35)	0.23(0.12; 0.55)	0.21(0.09; 0.36)	0.648	0.628	0.433	0.904
C20SA	0.02(0.01; 0.04)	0.02(0.01; 0.03)	0.02(0.01; 0.04)	0.747	0.911	0.805	0.509
doxSO	0.2(0.15; 0.7)	0.17(0.08; 0.44)	0.19(0.07; 0.78)	0.353	0.248	0.39	0.697
doxSA	0.1(0.07; 0.24)	0.09(0.03; 0.24)	0.07(0.03; 0.33)	0.238	0.208	0.211	0.762

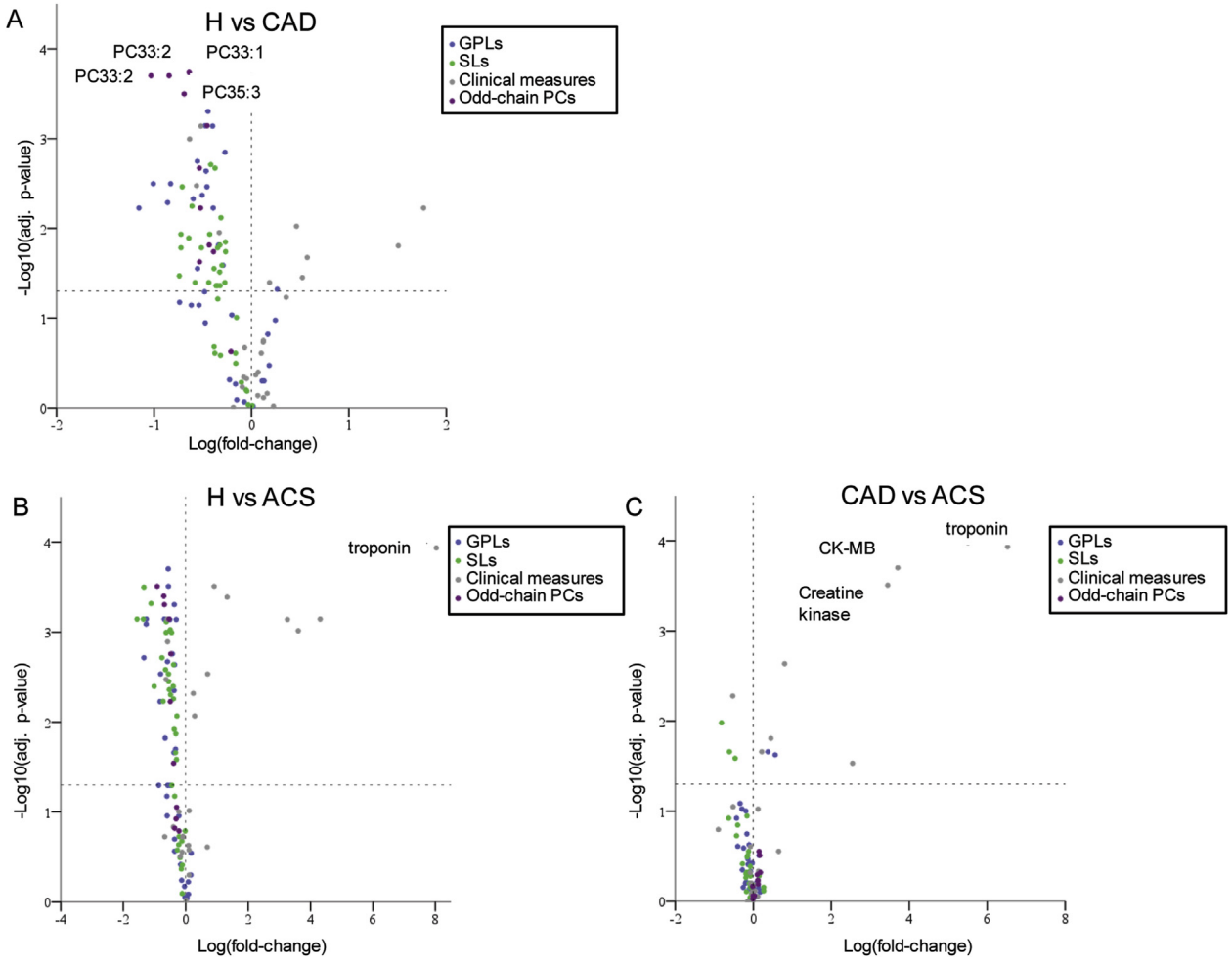

Fig. 1. Volcano plots illustrating the relative differences between healthy subjects and patients with CAD or AMI. The x axis reflects the change (displayed as -log(fold change) whereas the y axis reflects the significance of change (displayed as -log(adj. p value). p values were calculated by the Mann-Whitney U test. Colored dots represent the individual groups (blue, GPLs; green, SMs, Cers/HexCers, S1Ps and sphingoid bases; crimson, odd-chain PCs; grey, clinical measures). The p values were adjusted for multiple comparisons according to the Benjamini-Hochberg procedure. The dashed horizontal line reflects an adj. $p < 0.05$. (AMI, acute myocardial infarction; CAD, coronary artery disease; H, healthy subjects; PC, phosphatidylcholine; GPL, glycerophospholipids; SL, sphingolipid). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

Lipids	Total cholesterol	HDL-C	LDL-C
Total PC	0.81	0.66	0.68
PC32:0	0.58	0.65	0.45
PC32:1	0.49	0.51	0.42
PC33:1	0.72	0.8	0.57
PC33:2	0.63	0.71	0.5
PC33:3	0.57	0.75	0.43
PC34:0	0.64	0.59	0.52
PC34:1	0.57	0.64	0.44
PC34:2	0.68	0.5	0.6
PC34:3	0.64	0.61	0.54
PC35:1	0.72	0.57	0.66
PC35:2	0.66	0.61	0.56
PC35:3	0.66	0.68	0.52
PC35:4	0.44	0.45	0.36
PC35:5	0.48	0.54	0.39
PC36:0	0.55	0.42	0.46
PC36:1	0.73	0.53	0.68
PC36:2	0.55	0.33	0.49
PC36:3	0.72	0.43	0.62
PC36:4	0.13	0.1	0.12
PC36:5	0.27	0.48	0.17
PC37:4	0.48	0.38	0.43
PC37:5	0.44	0.56	0.3
PC37:6	0.53	0.61	0.43
PC38:3	0.42	-0.18	0.44
PC38:4	0.08	-0.16	0.1
PC38:5	0.33	0.35	0.24
PC38:6	0.1	0.01	0.07
PC40:5	0.29	0.11	0.25
PC40:6	0.06	-0.18	0.07
Total LPC	0.62	0.47	0.59
LPC16:0	0.81	0.53	0.74
LPC18:0	0.73	0.32	0.71
LPC18:1	0.28	0.46	0.26
LPC18:2	0.01	0.12	0.04
Total PE	0.54	0.2	0.48
PE34:2	0.56	0.26	0.5
PE36:4	0.45	0.2	0.38
PE36:3	0.54	0.29	0.49
PE36:2	0.6	0.2	0.54
PE36:1	0.47	0.06	0.45
PE37:5	0.51	0.53	0.36
PE37:4	0.38	0.36	0.32
PE38:6	0.29	0.1	0.23
PE38:5	0.4	0.26	0.34
PE38:4	0.4	0.07	0.37

Lipids	Total cholesterol	HDL-C	LDL-C
Total SM	0.77	0.59	0.7
SM32:1	0.73	0.65	0.65
SM34:0	0.59	0.54	0.5
SM34:1	0.8	0.63	0.71
SM34:2	0.69	0.6	0.63
SM36:1	0.56	0.33	0.54
SM36:2	0.51	0.32	0.5
SM38:1	0.63	0.47	0.55
SM39:1	0.66	0.42	0.66
SM40:1	0.62	0.34	0.59
SM40:2	0.64	0.53	0.6
SM41:1	0.81	0.46	0.75
SM41:2	0.59	0.57	0.56
SM42:1	0.65	0.33	0.63
SM42:2	0.6	0.57	0.52
SM42:3	0.41	0.41	0.37
Total Cer/HexCer	0.78	0.35	0.75
Cer(d18:1/24:0)	0.46	0.09	0.45
HexCer(d18:1/22:0)	0.76	0.27	0.75
HexCer(d18:1/23:0)	0.72	0.4	0.67
HexCer(d18:1/24:0)	0.76	0.34	0.75
HexCer(d18:1/24:1)	0.65	0.36	0.58
S1P species			
16:1-S1P	0.6	0.54	0.55
18:1-S1P	0.44	0.2	0.41
18:0-S1P	0.28	0.06	0.29
Sphingoid bases of sphingolipids			
C16SO	0.77	0.47	0.75
C16SA	0.8	0.26	0.8
C17SO	0.85	0.51	0.81
C18SO	0.93	0.6	0.86
C18SA	0.82	0.47	0.77
C18SAdiene	0.78	0.55	0.73
C19SO	0.63	0.08	0.7
C20SO	0.57	0.21	0.59
C20SA	0.47	-0.06	0.55
doxSO	0.35	0.14	0.31
doxSA	0.44	0.03	0.43

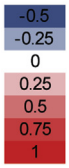

Fig. 2. Heat map of the spearman correlations between lipid species and cholesterol levels (total, HDL-C, LDL-C) over the entire cohort (N = 49). Red color indicates positive correlation, blue - negative. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

shown in Fig. 3D–F. PCs were more prominent in HDL (Fig. 3A). 55% of total PCs were found in the HDL, approximately 34% were found in the LDL, 9% were found in the LFF and 2% of PCs were found in the combined CM/VLDL fractions. Odd and even-chain PC species showed a similar distribution, with HDL being the major carrier of these lipids (Fig. 3D). For SM, 56% was found in LDL, 36% in HDL, 5% in LFF and 2% in CM/VLDL (Fig. 3B, E). 80% of the total LPC species were found in the LFF, indicating that plasma LPCs are primarily bond to albumin (Fig. 3C). Small amounts of LPCs were present in LDL (11%) and HDL (7%). A prominent difference in the lipoprotein distribution was seen for LPC18:0, when compared to other LPC species (Fig. 3F). The proportion of LPC18:0 was decreased by 20% in the LFF and increased by 11% in the LDL fraction relative to the proportion of LPC16:0.

4. Discussion

In our study we found significant differences in the lipid profile between healthy subjects and patients with stable CAD or AMI. In the CAD group, plasma concentrations of 45 glycerophospholipids and sphingolipid species as well as an atypical 16:1-S1P were significantly lower compared with healthy subjects. Also sphingolipids which are formed on a C_{17}SO, C_{18}SO, C_{18}SA and C_{18}SAdiene sphingoid base backbone were significantly lower. In the AMI group, 42 plasma lipid species were significantly reduced compared to healthy subjects. However, the observed differences were more related to CAD than AMI as only minor differences in the lipid profile were seen between the CAD and AMI group. The observed differences seem to be independent of statin use as we did not see a significant effect of a cholesterol lowering therapy (statins) on glycerophospholipids and sphingolipid levels.

Four odd-chain PC species (PC33:1, PC33:2, PC33:3 and PC35:3), identified as PC plasmalogens were the most significantly altered lipids between the healthy and the CAD/AMI group. Reduced plasmalogen plasma levels were reported previously for hypertensive and obese patients [25,26] as well as for patients with CAD [9]. Decreased levels of HDL-associated PE plasmalogens were reported in patients with low HDL-C levels and in acute-phase HDL

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

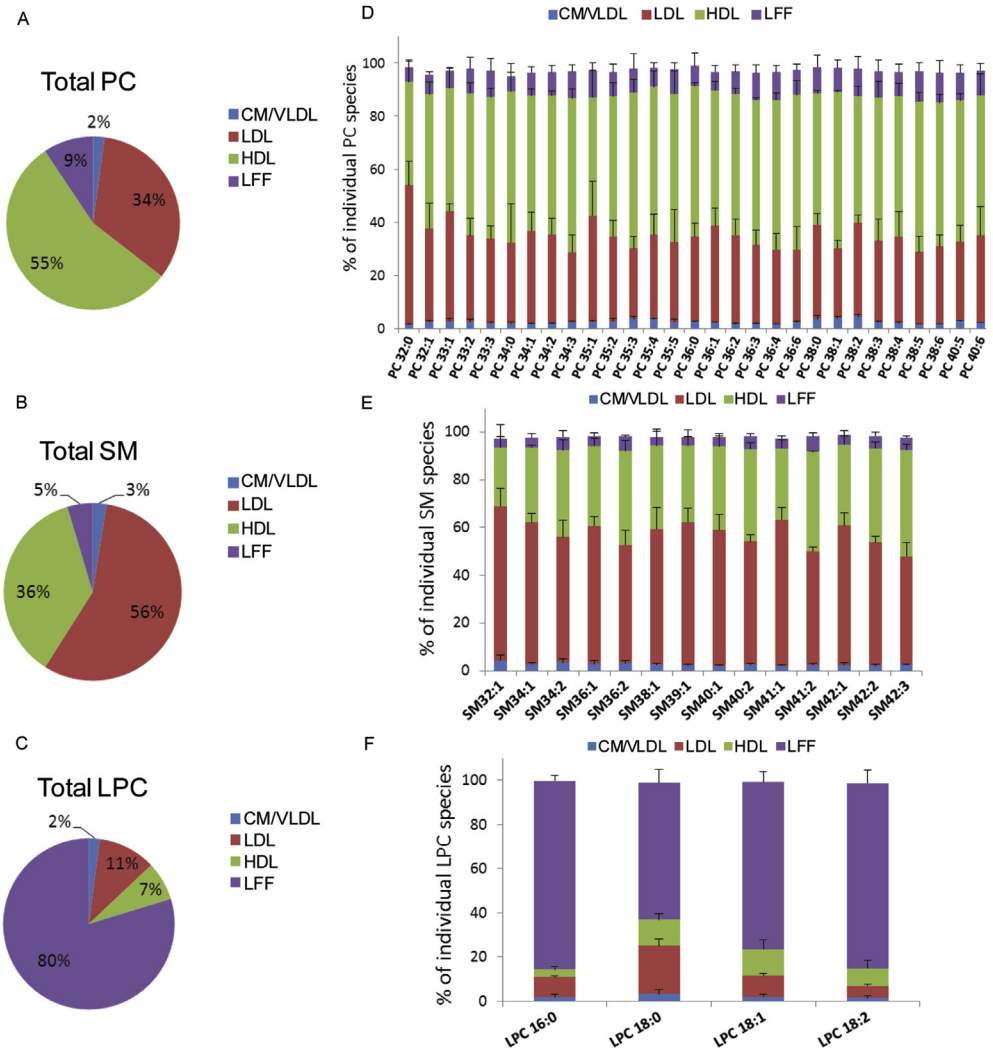

Fig. 3. Distribution of the total phosphatidylcholines, lysophosphatidylcholines and sphingomyelins (A–C) and of the individual species (D–F) in four major lipoprotein fractions. Percentages of total phosphatidylcholines (PCs), lysophosphatidylcholines (LPCs) and sphingomyelins (SMs) are expressed as mean (A–C) and the species specific distribution (mean ± SD) relative to the total lipoprotein content of the individual lipids (D–F). (CM/VLDL, chylomicron/very-low-density lipoprotein, HDL, high-density lipoprotein; LDL, low-density lipoprotein; LFF, lipoprotein-free fraction).

[11,27]. Comparing the individual lipoprotein fractions showed that PC33:1, PC33:2, PC33:3 and PC35:3 are largely associated with HDL which partly explains the strong positive correlations of these lipids with HDL-C as seen by us and others [28].

The physiological functions of plasmalogens are poorly understood. It is unclear whether decreased levels of PC plasmalogens are due to metabolic effects in CAD and AMI or whether plasmalogens play an active role in the pathogenesis of CAD. Plasmalogens possess antioxidant properties due to their ability to scavenge oxygen radicals [29]. Evidence from *in vitro* studies indicated that plasmalogens are capable of reducing the oxidation of cell membrane cholesterol, polyunsaturated fatty acids and LDL [30–32]. Thus, low levels of PC plasmalogens may be indicators of increased oxidative stress which may explain why PC plasmalogens, but not polyunsaturated PCs, were lower in patients with CAD and AMI.

Our analysis of the sphingoid base profile indicated a reduction of certain sphingoid bases in patients with CAD and AMI. This is in line with significantly reduced plasma levels of certain SM species in patients with CAD and AMI. However, 1-deoxyphingolipids (doxSA and doxSO), recently found as predictive biomarkers for the risk to develop type 2 diabetes mellitus [23,33] were not different between healthy controls and patients with CAD or AMI.

Our analysis also revealed the presence of various S1P species, including typical 18:1-S1P 18:1) and atypical 16:1-S1P, 17:1-S1P and 18:0 SA1P. Different S1P species were previously reported in plasma of healthy subjects [34], however, the existence of native 17:1-S1P was here reported for the first time. Among the phosphorylated sphingosines, the canonical S1P (18:1) was the most abundant and found to be lowered in AMI group. In contrast, 16:1-S1P, was diminished in plasmas of patients with CAD and AMI compared to healthy subjects. Also other studies reported reduced S1P levels in plasma and HDL of patients with CAD [22,35] as well as a significant inverse association of S1P (18:1) and SA1P (18:0) levels in the HDL-containing fraction of serum with the occurrence of ischemic heart disease [36]. In our study we did not see any significant association between plasma 18:0-SA1P levels and CAD or AMI. Canonical S1P (18:1) is an important regulator of vascular function as well as immune and inflammation responses. HDL is the major carrier of S1P in plasma and HDL bound S1P was reported to mediate several atheroprotective properties HDL [36], [37]. Therefore, differences in the concentration and composition of S1Ps in plasmas of healthy controls and patients with either acute or

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

chronic cardiovavascular disease may reflect differences in atheroprotective plasma functions.

4.1. Limitations

This study was designed as a purely explorative approach to identify novel lipid signatures in patients with CAD and AMI. Due to the small group sizes it was not possible to adjust for all potential confounders such as HDL-C, LDL-C, TG or medications without the risk of over fitting. This might introduce a bias in particular for the observed associations with HDL-C which is generally lower in the CAD patients. However, not all analyzed lipid species showed the same association with HDL-C indicating that changes in total HDL-C plasma levels are also associated with alterations in the lipid composition of HDL. Further lipid species might show significant changes when validating the study in a bigger cohort.

5. Conclusions and perspectives

In conclusion, we found that plasma levels of various glycerophospholipid and sphingolipid species are reduced in patients with CAD and AMI. Among them, PC33:1, PC33:2, PC33:3 and PC35:3, identified as plasmalogens PC(P-18:0/18:2), PC(P-18:0/16:0), PC(P-16:0/18:1) and PC(P-16:0/18:2), were the most significantly altered species in the plasma of patients with CAD. Reduced plasmalogen levels might be indicators for oxidative stress as it is typically seen in patients with CAD. We also found that these PC species are preferentially localized in HDL particles and correlate positively with HDL-C levels. However, as this was an explorative study to find novel lipid signatures the observed associations need to be validated in a larger cohort which allows to adjust for more potential confounders in multivariate statistical tests.

Disclosures

The authors have no conflict of interest pertinent to the subject of this study.

Funding

TH received a grant from the Zurich Center of Integrated Human Physiology, University of Zurich (ZIHP). AvE was supported by the Foundation for Pathobiochemistry and Molecular Diagnostics of the German Society for Clinical Chemistry and Laboratory Medicine. The SPUM-ACS study (RK, UL, DH, NR, FM, SW, CMM, TFL) was funded by the Swiss National Science Foundation (SPUM 33CM30-124112); Swiss Heart Foundation; Fondation Leducq and the Foundation for Cardiovascular Research, Zurich. The SPUM consortium was supported by Roche, Eli Lilly (USA), AstraZeneca, Medtronic, MSD, Sanofi, St. Jude Medical (all Switzerland).

Acknowledgments

The authors thank all physicians and nurses involved in the SPUM-ACS study and the participants for their contribution to the study and blood sample donation. The authors wish to thank Silvia Radosavljevic for the isolation of the lipoprotein fractions.

References

[1] M. Nichols, N. Townsend, P. Scarborough, M. Rayner, Cardiovascular disease in Europe 2014: epidemiological update, Eur. Heart J. 35 (42) (Nov 7 2014) 2929.
[2] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (7347) (May 19 2011) 317–325.
[3] F. Crea, G. Liuzzo, Pathogenesis of acute coronary syndromes, J. Am. Coll. Cardiol. 61 (1) (Jan 8 2013) 1–11.
[4] K. Ekroos, M. Janis, K. Tarasov, R. Hurme, R. Laaksonen, Lipidomics: a tool for studies of atherosclerosis, Curr. Atheroscler. Rep. 12 (4) (Jul 2010) 273–281.
[5] T. Hornemann, T.S. Worgall, Sphingolipids and atherosclerosis, Atherosclerosis 226 (1) (Jan 2013) 16–28.
[6] P. Wiesner, K. Leidl, A. Boettcher, G. Schmitz, G. Liebisch, Lipid profiling of FPLC-separated lipoprotein fractions by electrospray ionization tandem mass spectrometry, J. Lipid Res. 50 (3) (Mar 2009) 574–585.
[7] M. Dashti, W. Kulik, F. Hoek, E.C. Veerman, M.P. Peppelenbosch, F. Rezaee, A phospholipidomic analysis of all defined human plasma lipoproteins, Sci. Rep. 1 (2011) 139.
[8] S.M. Hammad, J.S. Pierce, F. Soodavar, et al., Blood sphingolipidomics in healthy humans: impact of sample collection methodology, J. Lipid Res. 51 (10) (Oct 2010) 3074–3087.
[9] P.J. Meikle, G. Wong, D. Tsorotes, et al., Plasma lipidomic analysis of stable and unstable coronary artery disease, Arterioscler. Thromb. Vasc. Biol. 31 (11) (Nov 2011) 2723–2732.
[10] M. Nishimukai, R. Maeba, A. Ikuta, et al., Serum choline plasmalogens-those with oleic acid in sn-2-are biomarkers for coronary artery disease, Clin. Chim. Acta 437 (Nov 1 2014) 147–154.
[11] W. Pruzanski, E. Stefanski, F.C. de Beer, M.C. de Beer, A. Ravandi, A. Kuksis, Comparative analysis of lipid composition of normal and acute-phase high density lipoproteins, J. Lipid Res. 41 (7) (Jul 2000) 1035–1047.
[12] C.E. Kostara, A. Papathanasiou, N. Psychogios, et al., NMR-based lipidomic analysis of blood lipoproteins differentiates the progression of coronary heart disease, J. Proteome Res. 13 (5) (May 2 2014) 2585–2598.
[13] I. Sutter, S. Velagapudi, A. Othman, et al., Plasmalogens of high-density lipoproteins (HDL) are associated with coronary artery disease and anti-apoptotic activity of HDL, Atherosclerosis 241 (2) (Aug 2015) 539–546.
[14] A. Kontush, M.J. Chapman, Lipidomics as a tool for the study of lipoprotein metabolism, Curr. Atheroscler. Rep. 12 (3) (May 2010) 194–201.
[15] C. Stegemann, I. Drozdov, J. Shalhoub, et al., Comparative lipidomics profiling of human atherosclerotic plaques, Circ. Cardiovasc Genet. 4 (3) (Jun 2011) 232–242.
[16] Y.A. Hannun, L.M. Obeid, Many ceramides, J. Biol. Chem. 286 (32) (Aug 12 2011) 27855–27862.
[17] A. Penno, M.M. Reilly, H. Houlden, et al., Hereditary sensory neuropathy type 1 is caused by the accumulation of two neurotoxic sphingolipids, J. Biol. Chem. 285 (15) (Apr 9 2010) 11178–11187.
[18] N.C. Zitomer, T. Mitchell, K.A. Voss, et al., Ceramide synthase inhibition by fumonisin B1 causes accumulation of 1-deoxysphinganine: a novel category of bioactive 1-deoxysphingoid bases and 1-deoxydihydroceramides biosynthesized by mammalian cell lines and animals, J. Biol. Chem. 284 (8) (Feb 20 2009) 4786–4795.
[19] H. Reiser, R. Klingenberg, D. Hof, et al., Circulating FABP4 is a prognostic biomarker in patients with acute coronary syndrome but not in asymptomatic individuals, Arterioscler. Thromb. Vasc. Biol. 35 (8) (Aug 2015) 1872–1879.
[20] C. Cavelier, L. Rohrer, A. von Eckardstein, ATP-Binding cassette transporter A1 modulates apolipoprotein A-I transcytosis through aortic endothelial cells, Circ. Res. 99 (10) (Nov 10 2006) 1060–1066.
[21] P. Haimi, A. Uphoff, M. Hermansson, P. Somerharju, Software tools for analysis of mass spectrometric lipidome data, Anal. Chem. 78 (24) (Dec 15 2006) 8324–8331.
[22] I. Sutter, R. Park, A. Othman, et al., Apolipoprotein M modulates erythrocyte efflux and tubular reabsorption of sphingosine-1-phosphate, J. Lipid Res. 55 (8) (Jun 20 2014) 1730–1737.
[23] A. Othman, M.F. Rutti, D. Ernst, et al., Plasma deoxysphingolipids: a novel class of biomarkers for the metabolic syndrome? Diabetologia 55 (2) (Feb 2012) 421–431.
[24] D. Yekutieli, Y. Benjamini, Resampling-based false discovery rate controlling multiple test procedures for correlated test statistics, J. Stat. Plan. Inference 82 (1–2) (Dec 1 1999) 171–196.
[25] J. Graessler, D. Schwudke, P.E. Schwarz, R. Herzog, A. Shevchenko, S.R. Bornstein, Top-down lipidomics reveals ether lipid deficiency in blood plasma of hypertensive patients, PLoS One. 4 (7) (2009) e6261.
[26] K.H. Pietilainen, M. Sysi-Aho, A. Rissanen, et al., Acquired obesity is associated with changes in the serum lipidomic profile independent of genetic effects–a monozygotic twin study, PLoS One. 2 (2) (2007) e218.
[27] P.P. Laurila, I. Surakka, A.P. Sarin, et al., Genomic, transcriptomic, and lipidomic profiling highlights the role of inflammation in individuals with low high-density lipoprotein cholesterol, Arterioscler. Thromb. Vasc. Biol. 33 (4) (Apr 2013) 847–857.
[28] R. Maeba, T. Maeda, M. Kinoshita, et al., Plasmalogens in human serum positively correlate with high- density lipoprotein and decrease with aging, J. Atheroscler. Thromb. 14 (1) (Feb 2007) 12–18.
[29] S. Wallner, G. Schmitz, Plasmalogens the neglected regulatory and scavenging lipid species, Chem. Phys. Lipids. 164 (6) (Sep 2011) 573–589.
[30] D. Reiss, K. Beyer, B. Engelmann, Delayed oxidative degradation of polyunsaturated diacyl phospholipids in the presence of plasmalogen phospholipids in vitro, Biochem. J. 323 (Pt 3) (May 1 1997) 807–814.
[31] G. Jurgens, A. Fell, G. Ledinski, Q. Chen, F. Paltauf, Delay of copper-catalyzed oxidation of low density lipoprotein by in vitro enrichment with choline or ethanolamine plasmalogens, Chem. Phys. Lipids 77 (1) (Aug 1 1995) 25–31.
[32] R. Maeba, N. Ueta, Ethanolamine plasmalogens prevent the oxidation of cholesterol by reducing the oxidizability of cholesterol in phospholipid bilayers, J. Lipid Res. 44 (1) (Jan 2003) 164–171.

Published in final edited form as: Atherosclerosis. 2016 Mar;246:130-40. doi: 10.1016/j.atherosclerosis.2016.01.003

[33] M. Bertea, M.F. Rutti, A. Othman, et al., Deoxysphingoid bases as plasma markers in diabetes mellitus, Lipids Health Dis. 9 (2010) 84.
[34] O. Quehenberger, A.M. Armando, A.H. Brown, et al., Lipidomics reveals a remarkable diversity of lipids in human plasma, J. Lipid Res. 51 (11) (Nov 2010) 3299–3305.
[35] K.J. Sattler, S. Elbasan, P. Keul, et al., Sphingosine 1-phosphate levels in plasma and HDL are altered in coronary artery disease, Basic Res. Cardiol. 105 (6) (Nov 2010) 821–832.
[36] K.M. Argraves, A.A. Sethi, P.J. Gazzolo, et al., S1P, dihydro-S1P and C24:1-ceramide levels in the HDL-containing fraction of serum inversely correlate with occurrence of ischemic heart disease, Lipids Health Dis. 10 (2011) 70.
[37] T.F. Luscher, U. Landmesser, A. von Eckardstein, A.M. Fogelman, High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target, Circ. Res. 114 (1) (Jan 3 2014) 171–182.